TRANSFORMATIONS OF SULPHUR AND ITS COMPOUNDS IN SOILS

Transformations du soufre et de ses composés dans le sol

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I. — INTRODUCTION : SULPHUR IN SOILS

As much as 90 p. 100 of the sulphur in soils may be present in organic compounds in plant and animal protein residues (FRENEY, 1967 ; SOKOLOVA and KARAVAIKO, 1968). The chemical status of sulphur in soils depends on the degree of aeration of the soil. For example, in aerobic, unwaterlogged Australian soils, FRENEY (1961, 1967) found that only 1 p. 100 of the sulphur was present in compounds less oxidized than sulphate. In poorly drained or anaerobic water logged soils considerable reduction of sulphate to sulphide can occur (STARKEY, 1966 ; ROY and TRUDINGER, 1970) with the subsequent precipitation of sulphide as pyrite or other metal sulphides. In moist soils considerable amounts of organically-bound sulphur may be present (STARKEY, 1966 ; FRENEY, 1967) and sulphide or sulphate may be generated from this under anaerobic or aerobic conditions respectively. Sulphide generation in soils can result in the soil becoming alkaline and producing net fixation of carbon dioxide from the atmosphere, this making a water logged soil more calcareous. Drainage of such sulphide rich soils frequently results in rapid sulphate production and a marked increase in acidity, sometimes producing pH values near pH 0.

Oxidation and reduction reactions of sulphur and its compounds in soils are mainly brought about by bacteria. The degree of aeration of the soil dictates whether oxidizing or reducing bacteria predominate and hence determines the proportions of sulphate and sulphide produced.

In untreated soils, little gross change in sulphur composition is generally observed (STARKEY, 1966), although a state of dynamic balance is no doubt maintained between sulphur content, microflora and plant cover (SIMON-SYLVESTRE, 1960 ;

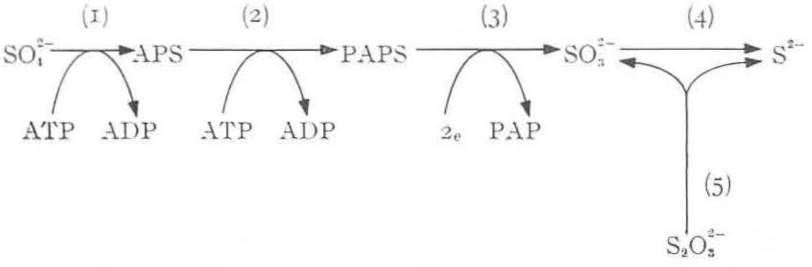
Footnote : Abbreviations used in the text : AMP, Adenosine 5′-monophosphate ; ADP, adenosine 5′-diphosphate ; ATP, adenosine 5′-triphosphate ; APS, adenosine 5′-phosphosulphate ; PAPS, 3′-phosphoadenosine 5′-phosphosulphate ; PPi, inorganic pyrophosphate ; Pi, inorganic orthophosphate.

STARKEY, 1966). The addition of sulphur or thiosulphates to a soil may, however, result in changes in the microflora, sulphur compounds present and in physical factors such as pH.

This paper is concerned principally with *a*) the bacteria responsible for oxidation and reduction of sulphur compounds ; *b*) the reactions they catalyze ; *c*) what is at present known about the biochemistry of these reactions ; *d*) the significance of intermediate sulphur compounds formed by, or because of, the bacteria ; and *e*) some effects of the bacteria on the soil environment.

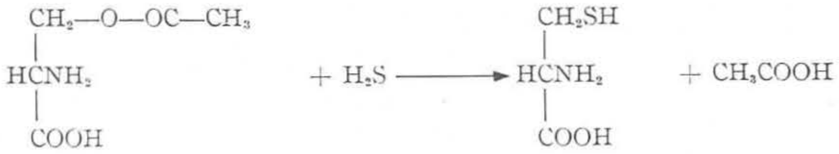
II. — ASSIMILATION OF SULPHUR

All soil organisms, microbes, plants or animals, need sulphur for cell synthesis and most can use sulphate as a principal source of this sulphur. For assimilation, sulphate is reduced metabolically to sulphide and converted to sulphur amino acids, such as cysteine and methionine (for reviews, see ROY and TRUDINGER, 1970 ; TRUDINGER, 1969). The reactions of assimilatory sulphate and thiosulphate reduction as are known to occur in microbes are out lined below :



Enzymes catalyzing the reactions are 1) ATP sulphurylase ; 2) APS kinase ; 3) PAPS reductase ; 4) assimilatory sulphite reductase (generally using $NADPH_2$ as a reductant) ; 5) thiosulphate reductase.

Sulphide is converted to cysteine by an enzymic reaction with serine or O-acetyl serine :

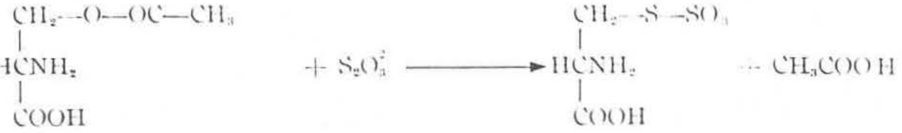


$$\begin{matrix} CH_2{-}O{-}OC{-}CH_3 \\ | \\ HCNH_2 \\ | \\ COOH \end{matrix} + H_2S \longrightarrow \begin{matrix} CH_2SH \\ | \\ HCNH_2 \\ | \\ COOH \end{matrix} + CH_3COOH$$

O-acetyl serine cysteine

The synthesis of other organic sulphur compounds, such as methionine, may proceed from cysteine.

Many microorganisms can use thiosulphate as a source of assimilable sulphur and both fungi and bacteria may form S-sulphocysteine from it by enzyme-catalysed reaction with serine or O-acetyl serine (ROY and TRUDINGER, 1970 ; CHAMBERS and TRUDINGER, 1969) :



$$\begin{array}{l} \mathrm{CH_2-O-OC-CH_3} \\ | \\ \mathrm{HCNH_2} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{S_2O_3^{2-}} \longrightarrow \begin{array}{l} \mathrm{CH_2-S-SO_3} \\ | \\ \mathrm{HCNH_2} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{CH_3COOH}$$

S-sulphocysteine

This compound is converted to cysteine in the presence of reduced glutathione.

Organic sulphur (principally as cystine, methionine and organic sulphates ; FRENEY, 1967) are liberated into soils on the death and lysis of the soil microflora as well as from dead animals and plant tissues.

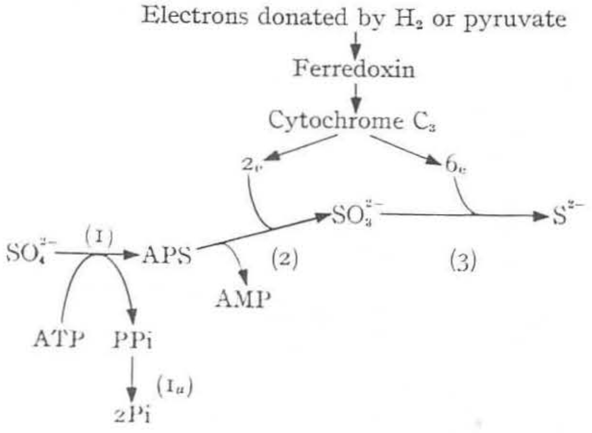
III. — SULPHATE REDUCTION AND SULPHIDE FORMATION IN SOILS

Sulphate-reducing bacteria

Detailed reviews concerning these bacteria have been published recently by PECK (1962), POSTGATE (1965), NICHOLAS (1967), TRUDINGER (1969), ROY and TRUDINGER (1970).

Large-scale sulphide generation is usually a property of bacteria carrying out *dissimilatory* sulphate reduction, where amounts of sulphate far in excess of the organisms requirements for cellular sulphur are reduced to sulphide by a respiratory electron transport system that uses sulphate instead of oxygen as an electron acceptor. Moreover these bacteria are generally strictly anaerobic, and will not grow in the presence of normal atmospheric concentrations of oxygen. Two principal genera are recognized : *Desulfovibrio* and *Desulfotomaculum* (CAMPBELL and POSTGATE, 1965), and both occur in waters, muds and soils (ROY and TRUDINGER, 1970). Various other sulphate-reducing species also exist (TRUDINGER and JONES, 1969).

Organic nutrients or hydrogen gas are used to supply electrons for sulphate reduction and the oxidation-reduction pigments ferredoxin (or flavodoxin) and cytochrome C_3 are electron carriers between hydrogen or pyruvate and the sulphate reduction pathway. This pathway in *Desulfovibrio* is outlined below :



Reaction (*1*) is effected by ATP sulphurylase, which has been isolated by several workers, and the reaction is drawn to APS synthesis *in vivo* by the hydrolysis of the other product, pyrophosphate, to orthophosphate by the pyrophosphatase enzyme (reaction (*a*)) in *Desulfovibrio* (PECK, 1962 ; ROY and TRUDINGER, 1970).

Reaction (*2*) is catalyzed by APS reductase, an enzyme which has been highly purified and is present in dissimilatory sulphate-reducing bacteria, but is apparently absent from assimilatory sulphate-reducing heterotrophs (PECK, 1962 ; ROY and TRUDINGER, 1970). An alternative reaction involving APS is catalyzed by ADP sulphurylase in *Desulfovibrio* :

$$\mathrm{APS} + \mathrm{Pi} \rightarrow \mathrm{ADP} + \mathrm{SO_4^{2-}}$$

The significance of this enzyme in *Desulfovibrio* is unknown.

Reaction (*3*) is the least well understood step in sulphate reduction and is catalyzed by sulphite reductase. Clearly several intermediate oxidation states for sulphur may exist in the reduction of sulphite and the overall reaction requires six hydrogen equivalents :

$$\mathrm{SO_3^{2-}} + 3\ \mathrm{H_2} \rightarrow \mathrm{S^{2-}} + 3\ \mathrm{H_2O}$$

The intermediary formation of thiosulphate, dithionate and trithionate have been reported during sulphite reduction (SUH, NAKATSUKASA and AKAGI, 1968 ; FINDLAY and AKAGI, 1969 ; KOBAYASHI, TACHIBANA and ISHIMOTO, 1969), although thiosulphate was probably not a product of the sulphite reductase enzyme. Most probably the compounds found at intermediate stages of sulphite reduction are transitory and remain bound to the sulphite reductase protein rather than being accumulated as free intermediates. The formation of disulphur monoxide (IVERSON, 1967) and small amounts of sulphur during sulphite reduction may results from the breakdown of such intermediates (ROY and TRUDINGER, 1970). The major observed product of sulphate reduction is thus sulphide although small amounts of the products may appear in natural environments. Sulphide is also produced by *Desulfovibrio* from the reduction of thiosulphate, tetrathionate and colloidal sulphur (POSTGATE, 1959) :

$$\mathrm{S_2O_3^{2-}} + 4\ \mathrm{H_2} \rightarrow 2\ \mathrm{HS^-} + 3\ \mathrm{H_2O}$$
$$\mathrm{S_2O^{2-}} + 9\ \mathrm{H_2} \rightarrow \mathrm{S^{2-}} + 3\ \mathrm{H_2S} + 6\ \mathrm{H_2O}$$

The transport of electrons from hydrogen to sulphite yields energy that is trapped as ATP by electron transport phosphorylation in *Desulfovibrio* (PECK, 1966) and menaquinone-6, a possible intermediate electron carrier, may be involved in these energy-linked reactions (WEBER, MATSCHINER and PECK, 1970).

Under anaerobic conditions in wet soils much of the sulphide released is precipitated as insoluble iron sulphide (STARKEY, 1966) and pyrite so formed may persist in some soils for long periods (HARMSEN, 1954). Sulphate reduction by *Desulfovibrio* and *Desulfotomaculum* may also produce a magnetic iron sulphide and copper sulphides (POSTGATE, 1965), and the possibility is being investigated that metal sulphide deposition may actually occur within or on the surface of the bacterial cells themselves (HALLBERG, 1970 ; Trudinger, personal communication). Other heterotrophic bacteria also reduce thiosulphate or tetrathionate, releasing sulphide and sulphite or thiosulphate respectively (POLLOCK and KNOX, 1943 ; KAPRALEK, PICHINOTY and RIEGROVA, 1968 ; ROY and TRUDINGER, 1970).

Sulphide formation from organic sulphur compounds

Cysteine may be hydrolysed by bacteria to yield sulphide under aerobic or anaerobic conditions, by the reverse of the reactions also possibly involved in cysteine biosynthesis : cysteine + water → serine + hydrogen sulphide. Vitolins and Swaby (1967, 1969) found H_2S production from cysteine in all the Australian soils tested by them. Under anaerobic conditions, H_2S is probably released also from other sulphur compounds like methionine or taurine, but aerobically the degradation of organic compounds yields sulphate.

IV. — OXIDATION OF SULPHUR AND ITS COMPOUNDS

The oxidation of sulphur in soils has been comprehensively reviewed by Burns (1967). Inorganic forms of sulphur available for microbial oxidation in soils include thiosulphate and polythionates as well as sulphides, elemental sulphur and occasionally thiocyanate, while organically bound sulphur may be present in a number of minor compounds as well as in sulphur amino acids (Starkey, 1950 ; Burns, 1967). All these reduced forms of sulphur are readily oxidized by bacteria and both heterotrophic and lithotrophic bacteria may be involved.

A. — *Oxidation of inorganic sulphur compounds*

1. *Oxidation by chemolithotrophic bacteria.*

Some chemolithotrophic (or autotrophic) bacteria use the oxidation of inorganic sulphur to supply energy for growth and can obtain all the carbon required for cell synthesis by fixing atmospheric carbon dioxide (Kelly, 1967 ; Peck, 1968 ; Rittenberg, 1969). Probably the most important sulphur-oxidizing bacteria in soils are the *Thiobacillus species* (Burns, 1967 ; Starkey, 1966 ; Swaby and Vitolins, 1968). The reactions carried out by these organisms are well documented, but although numerous studies have been made on the biochemistry of sulphur oxidation, the bacterial oxidation pathways are still not unequivocally resolved. Comprehensive reviews are available by Vishniac et Santer (1957), Peck (1962), Trudinger (1967 *a*, 1969), Kelly (1968), Jackson, Moriartyand Nicholas (1968), Hutchinson, Johnstone and White (1969) and Roy and Trudinger (1970).

Below are cited the principal reactions carried out by thiobacilli together with some discussion of recent information about sulphur metabolism in thiobacilli.

Oxidations effected by Thiobacillus *spp.*

$$2\ S + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4 \tag{1}$$

$$Na_2S_2O_3 + 2\ O_2 + H_2O \rightarrow H_2SO_4 + Na_2SO_4 \tag{2}$$

$$4\ Na_2S_2O_3 + 2\ O_2 + H_2O \rightarrow 2\ Na_2S_4O_6 + 4\ NaOH \tag{3}$$

$$2\ Na_2S_4O_6 + 7\ O_2 + 6\ H_2O \rightarrow 2\ Na_2SO_4 + 6\ H_2SO_4 \tag{4}$$

$$2\ KSCN + 4\ O_2 + 4\ H_2O \rightarrow (NH_4)_2SO_4 + K_2SO_4 + 2\ CO_2 \tag{5}$$

$$5\ S + 6\ KNO_3 + 2\ H_2O \rightarrow 3\ K_2SO_4 + 2\ H_2SO_4 + 3\ N_2 \tag{6}$$

$$5\ Na_2S_2O_3 + 8\ NaNO_3 + H_2O \rightarrow 9\ Na_2SO_4 + H_2SO_4 + 4\ N_2 \tag{7}$$

$$12\ FeSO_4 + 3\ O_2 + 6\ H_2O \rightarrow 4\ Fe_2(SO_4)_3 + 4\ Fe(OH)_3 \tag{8}$$

While gaseous oxygen is used for oxidations by aerobic bacteria, nitrate-oxygen can be used and the nitrate reduced to nitrogen gas (equations 6, 7) by several thiobacilli when oxygen is in low supply (WOLLEY *et al.*, 1962 ; HUTCHINSON *et al.*, 1969). Ferrous iron is oxidized by *T. ferrooxidans* under acid conditions, and pyrite may also be oxidized (pH 1-4) by combined chemical and bacterial attack by the following reaction sequence :

$2\ FeS_2 + 2\ H_2O + 7\ O_2 \rightarrow 2\ FeSO_4 + 2\ H_2SO_4$ (Chemical)
$4\ FeSO_4 + O_2 + 2\ H_2SO_4 \rightarrow 2\ Fe_2(SO_4)_3 + 2\ H_2O$ (*T. ferrooxidans*)
$Fe_2(SO_4)_3 + FeS_2 \rightarrow 3\ FeSO_4 + 2\ S$ (Chemical)
$2\ S + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$ (*T. thiooxidans* ; *T. ferrooxidans*).

While the end product of oxidation of sulphur by thiobacilli is principally sulphate, polythionates, and sometimes elemental sulphur, are also formed. Tetrathionate is often observed in *Thiobacillus* cultures (equation 3) and can be the sole oxidation product under conditions of high oxygen tension or in the presence of reagents that combine with thiol groups in the bacteria (TRUDINGER, 1964 *a*, 1965, 1967 *a* ; KELLY, 1968 ; ROY and TRUDINGER, 1970).

Trithionate and pentathionate are sometimes reported (VISHNIAC, 1952 ; KELLY and SYRETT, 1966 ; TRUDINGER, 1967 *a*). Growing culture of *T. neapolitanus* accumulate trithionate in the culture medium and subsequently oxidize this further only at a very slow rate. In cultures growing exponentially (2-3 hours generation times) tetrathionate accumulation from 40 mM thiosulphate was slight and none remained at the time of exhaustion of the thiosulphate (when logarithmic growth ceases abruptly). Accumulation of trithionate was, however, progressive, and reached a maximum concentration of about 9 mM. One-third of the total thiosulphate-sulphur added was thus converted to trithionate-sulphur, which subsequently supported virtually no further growth (KELLY, unpublished data). JONES and HAPPOLD (1961) showed that phosphate concentration and the ratio of Na : K in the medium determined whether trithionate or tetrathionate accumulated in *T. thioparus* cultures. Identical quantities of trithionate were, however, accumulated by *T. neapolitanus* cultured in media with 10.5 mM or 115.2 mM phosphate and having Na : K ratios of 5.55 : 1 or 0.51 : 1 (KELLY, unpublished data).

Recently a marine Thiobacillus has been shown to oxidize thiosulphate in two phases : the first phase results in tetrathionate accumulation during rapid growth, and its use during the second phase of slower growth (MURPHY, KORNFELD and TILTON, 1970).

Recent developments in the study of the biochemistry of these reactions are considered below.

Complete oxidation of inorganic sulphur compounds to sulphate by enzymes from Thiobacillus species.

While conflicting views still exist concerning the pathway of sulphur oxidation (TRUDINGER, 1969), enzymes have been characterized that together form a system capable of catalyzing the complete oxidation of sulphur, thiosulphate and other sulphur compounds. These enzymes and the reactions concerned are as follows :

Enzyme IA : Thiosulphate reductase (Peck, 1960)

$$S_2O_3^{2-} + 2e^- + 2\ H^+ \rightarrow SO_3^{2-} + H_2S$$

Enzyme IB : Rhodanese (Roy and Trudinger, 1970)

$$CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$$

Rhodanese may also act as a thiosulphate reductase with a suitable electron donor, such as dihydrolipoate.

Enzyme IC : Thiosulphate-cleaving enzyme (postulated by Charles and Suzuki, 1966).

$$S_2O_3^{2-} \rightarrow S + SO_3^{2-}$$

This might be the reaction catalysed *in vivo* by the rhodanese enzyme.

Enzyme II : Sulphur-oxidizing enzyme (Suzuki and Silver, 1966; Roy and Trudinger, 1970)

$$S + O_2 + H_2O \rightarrow H_2SO_3$$
$$(\text{OR } S_8 + 8\ O_2 + 8\ H_2O \rightarrow 8\ H_2SO_3)$$

Enzyme III : Sulphite cytochrome *C* oxidoreductase (sulphite oxidase ; Charles and Suzuki, 1966 *b* ; Trudinger, 1969 ; Lyric and Suzuki, 1970 *a*)

$$SO_3^{2-} + 2\ \text{cytochrome}\ C\ Fe^{3+} + H_2O \rightarrow SO^{2-} + 2\ \text{cytochrome}\ C\ Fe^{2+} + 2H^+$$

(cytochrome *C* Fe^{2+} reoxidation by oxygen is catalysed by a cytochrome oxidase).

Enzyme IV : Adenosine 5′-phosphosulphate reductase (Peck, 1960; Bowen *et al.*, 1966 ; Lyric and Suzuki, 1970 *b*)

$$SO_3^{2-} + AMP \rightarrow APS + 2e^-$$

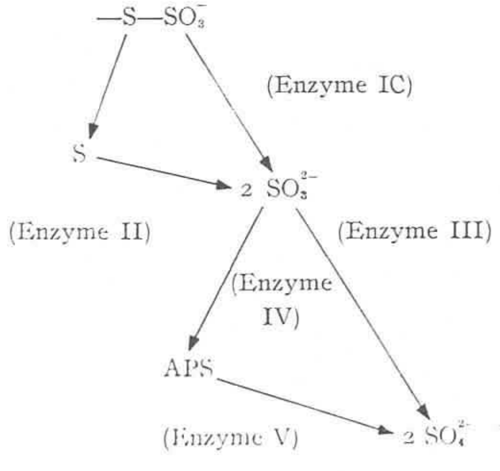
Enzyme V : ADP sulphurylase (Peck, 1970)

$$APS + Pi \rightarrow ADP + SO_4^{2-}$$

(AMP for enzyme IV can be regenerated by adenylate kinase in the bacteria :

$$2\ ADP \rightarrow ATP + AMP)$$

Complete oxidation of thiosulphate (or sulphur) is thus possible by this reaction sequence (Charles and Suzuki, 1966 *a* ; Lyric and Suzuki, 1970 *c*) :



The reactions of this scheme about which least are known are those affecting thiosulphate cleavage, and those converting sulphur to sulphite. If sulphide rather than sulphur is a product of thiosulphate cleavage, the sulphur-oxidizing enzyme might more properly be a sulphide-oxidizing enzyme, unless sulphide is first converted to sulphur by a sulphide oxidase (Peck, 1960). This may be unlikely as recent work by Moriarty (1969) and Moriarty and Nicholas (1969, 1970) shows that sulphide is oxidized by enzymes of *T. concretivorus* to form sulphate with the intermediate formation of long-chain sulphur compounds which are probably enzyme bound. The initial phase of sulphide oxidation seems to result in chains of atoms in which most of the sulphur is formally at the oxidation level of elemental sulphur. These chains of atoms are presumably the substrate for the sulphur-oxidizing enzyme in the progressive production of sulphite. The mode of addition of oxygen is uncertain, although probably oxygen from both the atmosphere and from water enter into combination with the sulphur. Probably no free intermediates are released during the oxidative steps between sulphur and sulphite. Mechanisms for oxidations of these sulphur chains have been discussed by Lees (1960), Trudinger (1967 *a*) and Kelly (1968). It seems likely that similar oxidation mechanisms exist in all the thiobacilli. At present, however, some workers find that the oxidation of elemental sulphur by disrupted bacteria is catalysed by soluble cell components (Suzuki and Silver, 1966), others find that a membrane system is involved (Adair, 1966) or that both soluble and particulate factors are needed (Kodama, 1969). Quite probably the systems are similar and the differences found are a result of the damage done to the membrane systems during cell breaking (Roy and Trudinger, 1970). It is uncertain whether sulphite is oxidized mainly by the APS pathway or the sulphite oxidase reaction, or both. This may depend on the strain of *Thiobacillus* used or on environmental factors.

Polythionate formation and metabolism are not accounted for by these reactions. Only one polythionate-forming enzyme has been purified : the thiosulphate-oxidizing enzyme, which is present in several thiobacilli (Trudinger 1961 ; Lyric and Suzuki, 1970 *c* ; Roy and Trudinger, 1970) and catalyzes this reaction :

$$2\ S_2O_3^{2-} + 2\ Fe\ (CN)_6^{3-} \rightarrow S_4O_6^{2-} + 2\ Fe\ (CN)_6^{4-}$$

Cytochromes may also act as electron acceptors for the oxidation. The role of this enzyme is uncertain, but Vishniac and Trudinger (1962) suspected that its physiological function might not be to produce tetrathionate but rather to act as an « activating » enzyme, perhaps prior to thiosulphate cleavage (Trudinger, 1967 *a*; Kelly, 1968).

Accumulation of tetrathionate, trithionate and other polythionates (London, 1964 ; London and Rittenberg, 1964) might result if rate-limiting steps in the prime reaction pathway cause alternative polythionate-producing reaction to occur, perhaps releasing polythionates from the enzyme-bound polysulphur chain (Lees, 1960 ; Kelly, 1968). Trithionate formation by *T. neapolitanus* (see above) seems such a regular process that it may be a normal, controlled part of the overall process of thiosulphate oxidation. The simplest mechanism postulated for trithionate formation depends on the oxidative condensation of sulphite and thiosulphate (Kelly and Syrett, 1966).

Polythionate oxidation is most likely to proceed only after they have been hydrolyzed or cleaved respectively to sulphite, thiosulphate and other smaller molecules (TRUDINGER, 1964 *b* ; OKUZUMI, 1966 ; ROY and TRUDINGER, 1970). It seems unlikely that a direct pathway from sulphur to sulphate via polythionate exists.

2. *Oxidation by photolithotrophic bacteria.*

These bacteria oxidize inorganic sulphur compounds in the *light* and do not develop in the presence of atmospheric oxygen concentrations (PFENNIG, 1967). They are important sulphur oxidizers in muds and lake waters, and possibly in flooded soils such as rice fields, but probably do not play an important role in aerobic soils (SWABY and VITOLINS, 1968 ; VITOLINS and SWABY, 1967, 1969).

3. *Oxidation by heterotrophic bacteria.*

Heterotrophic bacteria grow using organic compounds as sources of carbon and energy, but many seem able to oxidize inorganic sulphur compounds such as thiosulphate, sulphur and sulphite to polythionates, sulphur and sulphate (GLEEN and QUASTEL, 1953 ; PECK, 1962 ; BURNS, 1967 ; SWABY and VITOLINS, 1968 ; ROY and TRUDINGER, 1970). Little is known of the mechanism of these oxidations, except that TRUDINGER (1967 *b*) demonstrated a thiosulphate-oxidizing enzyme similar to the tetrathionate-forming enzyme in thiobacilli in each of two soil organisms. These organisms produced tetrathionate from thiosulphate. Thiocyanate is also oxidized to sulphate, and some of the sulphur is assimilated by at least one heterotrophic *Pseudomonas* (STAFFORD and CALLELY, 1969).

The quantitative importance of heterotrophic bacteria in inorganic sulphur oxidation is difficult to assess, although VITOLINS and SWABY (1967, 1969) think their contribution may in some cases be at least as important as that of the thiobacilli.

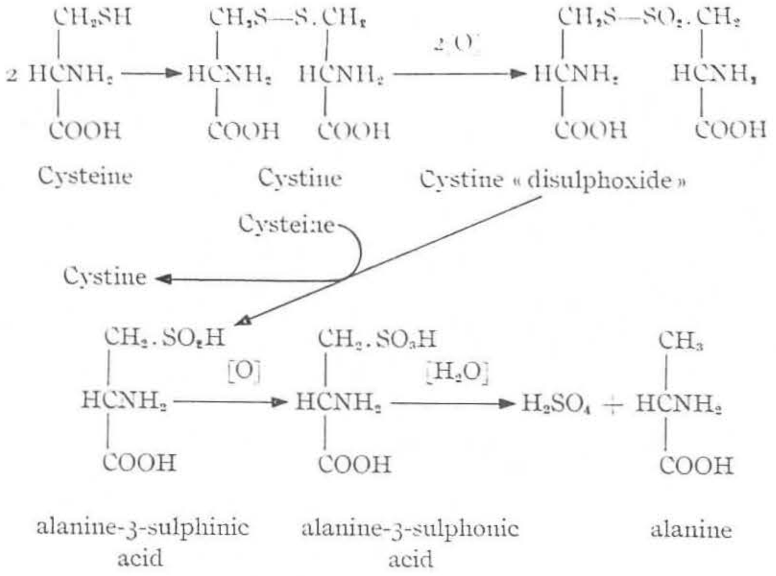
B. — *Oxidation of organic sulphur compounds*

Organic sulphur compounds are oxidized by heterotrophic bacteria and fungi in soils and the ultimate end product is usually sulphate, although polythionates and sulphur may also occur (STARKEY, SEGAL and MANAKER, 1953 ; FRENEY, 1967). The latter would be ready substrates for further oxidation by thiobacilli.

Little literature appears to be available on the mechanism of degradation in soils of specific organic sulphur compounds. Sulphide liberated from cysteine by the microbial cysteine desulphydrase may be oxidized by thiobacilli and heterotrophs, but probably the sulphur in many organic sulphur compounds is oxidized while still organically bound (ROY and TRUDINGER, 1970). Cysteine probably undergoes oxidation by the following reactions (p. 226), catalyzed by soil bacteria (ROY and TRUDINGER, 1970).

The possibility has, however, not been exclued that sulphur dioxide or sulphite ion might arise directly from alanine-3-sulphinic acid and then subsequently be oxidized to sulphate.

Cystine and methionine undergo fairly ready degradation in soils (BURNS, 1967 ; FRENEY, 1967).



V. — FACTORS AFFECTING SULPHUR TRANSFORMATIONS IN SOILS

For sulphur oxidation or reduction reactions to proceed the necessary microorganisms must obviously be present. It seems that most soils contain sulphate-reducing bacteria, even if they do not appear active in reducing sulphate in aerated soils (Postgate, 1965 ; Vitolins and Swaby, 1967). The majority of world soils examined contain at least one type of sulphur-oxidizing *Thiobacillus* (Starkey, 1935 ; Burns, 1967 ; Vitolins and Swaby, 1967, 1969) and heterotrophic bacteria capable of some sulphur compound oxidation or reduction are doubtless ubiquitously distributed.

The numbers of sulphur-metabolizing organisms in soils vary greatly. While the observable numbers of microbes in a soil may be as great as 2.5×10^{10} per gram of soil (Mishutin, Nikitin and Vostrov, 1968), the numbers of active *Thiobacillus* organisms may be less than a million (Attoe and Olson, 1966). Addition of sulphur fertilizers (sulphur or thiosulphate) may greatly increase the *Thiobacillus* population of a soil, even resulting in large, active bacterial population when *no* organism *seemed* to be present before soil supplementation (Gleen and Quastel, 1953 ; Burns, 1967). Some soils previously shown to be poor oxidizers of sulphur compounds were considerably improved by « seeding » with preparations containing active sulphur-oxidizers like Thiobacilli (Freney, 1967 ; Vitolins and Swaby, 1967, 1969). This practice could benefit soils genuinely lacking sulphur-oxidizing organisms, but most probably supplementing sulphur-poor soils with sulphur, polysulphides or thiosulphates would result in the natural development of vigorous populations of sulphur-oxidizing bacteria.

Fluctuations in populations of sulphate-reducing or oxidizing bacteria also accompany flooding and drainage of soils. An extreme example of this occurs as an annual cycle in delta soils in Southern India, where sulphate reduction during the period when the soils are flooded by sea water results in a concentration of elementary sulphur in the soil as high as 35 p. 100. This sulphur is quite readily oxidized when the soil drains in the dry season and soil pH falls from pH 7 to pH 3 (SUBBA RAO, 1951, quoted by FRENEY, 1967).

In partially flooded environments, sulphate reduction and formation may proceed simultaneously at different levels within the soil. In some bogs, for example, sulphide generated in the anaerobic depths is oxidized, presumably by bacteria, in the upper aerobic levels, and a seasonal rise and fall in the depth at which sulphide is detected is probably correlated with the depth at which anaerobiosis is maintained and the population of sulphate-reducing bacteria is maximal (CLYMO, 1965).

Addition of sulphur compounds to soils may greatly alter the physico-chemical state of the soils : the specific alterations depend largely on what type of bacterial reaction is favoured in the particular soil. Thus sulphur oxidation by *Thiobacillus* in aerobic soils may reduce them to pH 3 or even to acidities of pH 0. *T. thiooxidans* can produce sulphuric acid to give pH 0.7 and itself survive at pH 0.01 (KEMPNER, 1966). In contrast, large scale conversion of thiosulphate to tetrathionate in an aerobic soil by heterotrophs could produce an increase in alkalinity. Tetrathionate would probably not persist long in a soil, but would be further oxidized to sulphate by lithotrophs and heterotrophs (GLEEN and QUASTEL, 1953). Sulphate-reducing bacteria also produce alkaline conditions :

$$Na_2SO_4 + Fe(OH)_3 + 9\,[H] \rightarrow FeS + 2\,NaOH + 5\,H_2O$$

Two consequences of their activity are (*a*) the removal of essential metal nutrients like iron, by precipitation as insoluble sulphides, and (*b*) formation of carbonates by reaction of the NaOH with atmospheric carbon dioxide.

Further environmental factors regulating bacterial activity in soils were discussed by FRENEY (1967) and TISDALE and NELSON (1966). The principal factors are summarised below.

1. — *Water.*

Optimum bacterial activity occurs in high humidity and the organisms live in surface moisture films. Desiccated soils are thus metabolically inactive.

2. — *Temperature.*

Generally bacteria are most active at temperatures between 10°-40°C, with little sulphur oxidation occurring below 10° or above about 60°.

3. — *pH.*

Most microbes grow best between pH 6-8, although pH 10 is tolerated by some and different thiobacilli tolerate acidities down to minima of pH 0.01 (*T. thiooxidans*) to pH 4.5 (*T. thioparus*). Correct E_h is critical in the successful development of dissimilatory sulphate reducers.

4. — *Particle size.*

Soil organisms probably tend generally to adsorb on to soil particles and a fine particulate soil might support a larger stable microflora than one containing large dense crumbs. Similarly finely ground sulphur is more readily attacked than large coarse grains. Soil perfusion experiments (GLEEN and QUASTEL, 1953) show that the soil bacteria remain within a soil body through which liquid is passed. *Thiobacillus neapolitanus* may be cultured in a column of pure, washed sand through which a thiosulphate-culture medium is passed continuously. Very large populations of *Thiobacillus* develop, attached to the sand grains and few organisms tend to wash out with the passage of the perfusate medium (CHAMBERS, KELLY and TRUDINGER, unpublished data).

5. — *Nutrients.*

Supplies only of inorganic nitrogen salts, air and a few trace metals are needed by thiobacilli, in addition to oxidizable sulphur. Generally ample organic matter is available in arable of flooded or forested soils for the development of heterotrophic bacteria, including the sulphate-reducing ones.

6. — *Soil type.*

As a result of differences presumably in the factors already listed, different soils support different gross microbial populations (MISHUSTIN *et al.*, 1968), although their populations of specific sulphur bacteria would be governed only by the environmental factors influencing those groups of organisms.

IV. — CONCLUDING REMARKS

Laboratory and field studies clearly show that numerous inorganic forms of sulphur and the sulphur amino acids are readily degraded to sulphate or sulphide by soil microorganisms. Many of the factors influencing microbial activity in soils may be defined and a great deal is known of the biochemistry of the metabolism of sulphur compounds in isolated bacteria. Much is still, however, to be learned of the factors affecting the rate of sulphur oxidation by bacteria in soils and of the influence upon each other of the different types of bacteria developing in intimate association with each other, adsorbed to soil particles.

RÉSUMÉ

TRANSFORMATION DU SOUFRE ET DE SES COMPOSÉS

1. — *Le soufre dans les sols*

Les formes organiques et inorganiques du soufre sont oxydées et réduites par les bactéries du sol pour donner des sulfates, du soufre, des polythionates ou des sulfures, ceci selon la quantité d'oxygène disponible. Ce rapport étudie les types de bactéries et les réactions qu'elles ca-

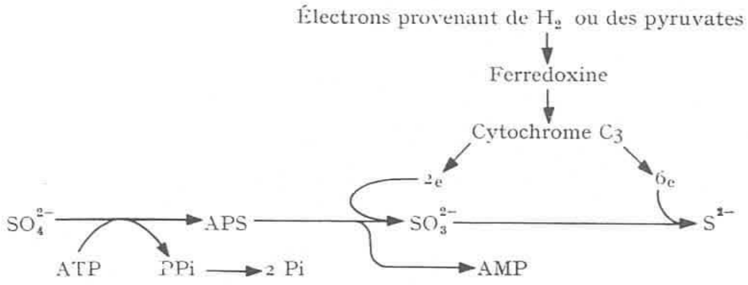
lysent dans les sols, et donne un aperçu de l'état actuel de nos connaissances sur les réactions biochimiques, et sur l'influence des transformations bactériennes du soufre sur l'environnement du sol.

2. — *Assimilation du soufre*

Nous décrivons dans ce paragraphe la réduction des sulfates assimilables, ainsi que les réactions conduisant la synthèse de la cystéine et de la S-sulphocystéine chez les microbes.

3. — *Réduction des sulfates et formation des sulfures par les bactéries dans les sols*

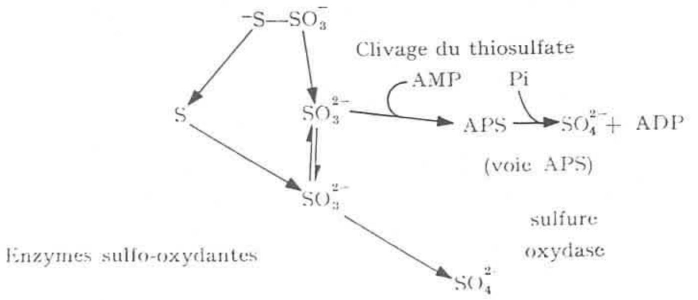
Nous traitons ensuite de la production de sulfures par respiration dissimilatrice et sulfato-réductrice des bactéries anaérobies *Desulfovibrio* et *Desulfotomaculum*. La progression et le cheminement des réactions de transferts d'hydrogène et de réduction des sulfates peuvent être ainsi schématisés :



Nous avons également indiqué les enzymes catalysant la production de sulfures à partir de la cystéine et la formation de sulfures métalliques insolubles résultant de l'activité bactérienne.

4. — *L'oxydation du soufre et de ses composés*

Dans cette partie, nous exposons en détail les phénomènes de l'oxydation du soufre, des thiosulfates, des sulfures et des polythionates par les bactéries lithotrophes et hétérotrophes. De nombreuses réactions catalysées par des thiobacilli sont aussi décrites, y compris celles concernant l'oxydation des pyrites en sulfate ferreux et en acide sulfurique. Nous donnons également un aperçu de la formation de polythionate par les thiocacilli et par les bactéries hétérotrophes. Une partie de l'exposé est également consacrée aux enzymes catalysant l'oxydation des composés du soufre chez les thiobacilli, et le schéma suivant explique les mécanismes d'oxydation des thiosulfates ou du soufre :



Nous avons également mentionné l'oxydation des composés organiques du soufre, tels que la cystéine, en sulfates par les organismes hétérotrophes du sol, et indiqué le mécanisme le plus probable intervenant dans la « décomposition » de la cystéine.

5. — *Facteurs susceptibles de modifier la transformation du soufre dans le sol*

Les principaux facteurs susceptibles d'influencer le type, l'activité et le nombre de bactéries sulfo-oxydantes et sulfo-réductrices sont les suivants :

a) degré d'aération ;
b) disponibilité du soufre ;
c) besoin en eau ;
d) effets de la température du pH et de l'E_h ;
e) influence de la taille des particules sur la taille de la « population » du sol et la vitesse d'attaque du soufre ou des composés minéraux ;
f) teneur en éléments nutritifs et types de sol.

Enfin, l'accent est mis sur l'influence profonde de l'activité des bactéries sulfo-oxydantes et sulfo-réductrices sur le bilan et les transformations du soufre dans les sols et sur les modifications considérables des propriétés physico-chimiques d'un sol pouvant résulter d'une addition de soufre ou d'une variation de son degré d'aération.

NOTA.

Abréviations :

AMP, adenosine 5'-monophosphate ;
ADP, adenosine 5'-diphosphate ;
ATP, adenosine 5'-triphosphate ;
APS, adenosine 5'-phosphosulfate ;
PAPS, 3'-phosphoadenosine 5'-phosphosulfate ;
PPi, pyrophosphate inorganique ;
Pi, orthophosphate inorganique.

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DISCUSSION

L. P. JONES (Great Britain).

I should like to ask Dr. Kelly wether there is any evidence of dissimilatory sulfate-reducing bacteria incorporating sulfides other than of iron in their cells. I am especially interested to hear about copper and lead sulfides in this context and also to hear about the latest thinking on the subject of the role of bacteria in the genesis or formation of sulphide minerals such as galena.

D. P. KELLY.

There is a body of evidence that suggests that sulphate-reducing bacteria may have produced at least some of the sulphide at present found in sedimentary ore bodies, containing metals such as copper, lead, zinc and silver in the sulphide form. This evidence is from studies of the ratios of the stable isotopes of sulphur in bacterially produced sulphides and in ore bodies. Stratified deposition of metal sulphides (including galena) have been produced in the laboratory using bacterially generated sulphide that was allowed to diffuse through gels and glass-bead layers to meet diffusing metal ions such deposition occurs *outside* the bacterial cell. Deposition in, or on the cell has been shown for iron sulphides and possibly for copper sulphides, but as far as I know, not for galena or other lead minerals. Metal concentrations to levels above those in their environment has been shown using many bacteria but this would not necessairly mean that metal sulphide precipitation in, or near the bacteria is anything other than an inorganic process.

G. SIMAN (Sweden).

I would like to ask Dr. Kelly, firstly, which do the heterotrophic microorganisms prefer : SO_4-S or organic sulphur in their assimilation processes : Did your experiments give any indication in this question ?

Secondly, what are your views on the end product of microbial mineralization of organic sulphur compounds, yielding SO_4-S ? Is the oxidative pathway of S^{--} included in the process of mineralization, or does it come separatly afterwards ?

D. P. KELLY.

Most, if not all, heterotrophic microorganisms prefer organic sulphur, as sulphur-amino-acids (methionine or cystine) rather than sulphate as a source of assimilable sulphur. Using S_{35} labelled sulphate, bacteria can generally be shown to incorporate unlabelled sulphur-amino-acids or more reduced inorganic forms of sulphur, such as sulphide or thiosulphate, in preference to at least part the sulphate S they would consume in the absence of alternative sulphur sources. To answer Dr. Siman's second question, I would say that probably microbial degradation of organic sulphur, say of cystine, probably results both in the direct production of sulphite or sulphate as shown by Freney, and in the production of sulphide which is further oxidized by thiobacilli and by inorganic oxidation. The relative contribution of the two mechanisms may depend on conditions prevailing in any given soil.